# The effect of an induced copper deficiency on the total plasma copper and unconjugated plasma progesterone concentrations during the oestrous cycle of the ewe

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The effect of a copper deficiency on certain aspects of reproduction in ewes was investigated. An effective copper deficiency was induced by using the copper antagonists cadmium, calcium and sulphate. An average decline in the plasma copper concentration from 160 µg/dl to 56 µg/dl was achieved. Further indications of the effectiveness of the copper deficiency were the loss of crimp in the wool and the decline in the average liver copper concentration from 131 µg/g to 34 µg/g dry matter. Plasma copper and progesterone concentrations were determined on days 1, 5, 8, 12 and 16 (day of oestrous) of two consecutive oestrous cycles. A rise in plasma copper was found which reached a maximum at the time when the progesterone concentration was at its highest (day 12 of the oestrous cycle). A subsequent decline in the copper concentration followed and a minimum was reached at the time of oestrus, the period when progesterone concentration is at a minimum. Therefore it seems that the need for copper is higher for reproduction than for normal body maintenance.

Die effek van 'n kopertekort op sekere reproduksie-aspekte by die ooi, is ondersoek. 'n Effektiewe kopertekort is by 'n groep ooie geïnduseer met behulp van die koperantagoniste kadmium, kalsium en sulfaat. 'n Gemiddelde verlaging van 160 µg/dl na 56 µg/dl is in die plasmakoperkonsentrasie verkry. Die effektiwiteit van hierdie kopertekort is verder weerspieël deur die verlies aan wolkarteling tydens hierdie periode asook die verlaging van die lewerkoperkonsentrasie vanaf gemiddeld 131 µg/g na 34 µg/g droë massa. Die plasmaprogesteroonkonsentrasie is op vasgestelde tye nl. dae 1, 5, 8, 12 en 16 (dag van estrus) van twee agtereenvolgende estrussiklusse, bepaal en daar is gevind dat 'n styging in die plasmakoperkonsentrasie voorkom wat 'n maksimum bereik tydens die periode wanneer progesteroonproduksie die hoogste is. 'n Daling in die plasmakoperkonsentrasie vind plaas en 'n minimum word bereik tydens estrus, die periode wanneer die progesteroonkonsentrasie op sy laagste is. Hiervolgens wil dit dus voorkom of die koperbehoeftes vir reproduksie hoër is as vir normale liggaamsonderhoud.

**Keywords:** Ewes, copper deficiency, plasma copper, plasma progesterone

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## Introduction

Copper deficiencies in ruminants and the consequent losses in production and reproduction are due to several factors. Low copper contents of pastures are known to occur in certain areas (Underwood, 1977), and the presence of mineral antagonists of copper such as cadmium, molybdenum, iron and zinc is accepted as an underlying interaction leading to copper shortages in grazing ruminants.

In cases where a copper deficiency exists, reproductive performance is impaired by decreased conception rates, while suboestrous and anoestrous conditions occur frequently (Mahadevan & Zubairy, 1969). Tsou, Daily, McLanahan, Parent, Tindall & Neill (1977) induced ovulation in the rabbit by intravenous administration of copper ions. Ovulation was preceded by a sharp rise in gonadotropin secretion, followed by an increase in luteinizing hormone levels which remained high for up to 6 h after treatment. Sato & Henkin (1973) found a relationship between plasma copper and oestrogen concentrations during the time of oestrous in the rat, but not during pregnancy. Although no specific interrelationship between progesterone and copper was found, the normal pattern of an increasing hormone concentration during pregnancy, was accompanied by an elevation of the copper concentration in the liver.

Considerable differences in the blood copper content are seen between various sheep breeds (Wiener, Field & Wood, 1969) and this is reflected in the variation in susceptibility of different breeds to conditions such as enzootic ataxia (Wiener, 1966). Differences in plasma copper concentrations between animals within the same breed are apparently the result of differences in haemoglobin type (Wiener, Hall & Hayter, 1973). According to Wiener, *et al.*, (1973), sheep belonging to haemoglobin type BB (genotype $Hb^{bb}$) show the highest blood copper content, followed by haemoglobin type AB (genotype $Hb^{ab}$) and lastly, haemoglobin type AA (genotype $Hb^{aa}$). The frequency of the various Hb types, especially $Hb^{aa}$ and $Hb^{bb}$, has a significant influence on the average blood copper concentration, thus the copper status of a herd is dependant on several factors and may be affected in a number of ways.

According to Maddox (1973), a connection exists between copper and the production rate of at least some of the reproductive hormones (Maddox, 1973). Although there are indications that progesterone may not be involved in this relationship in rats, the present study was, nevertheless, undertaken to re-examine the possibility of such a connection during the oestrous cycle of the ewe.

## Materials and Methods

Twenty SA Mutton Merino ewes (two-tooth) were divided into two groups of approximately equal average body mass (50,9 kg and 49,92 kg). The animals were housed in a feeding shed and treated as two separate groups. Ten wethers of the same age and breed were also included in the experiment. Six were slaughtered at the start of the experiment whereas the remaining four were placed with the experimental group. These four animals were slaughtered at the end of the first phase of the experiment. The livers of the slaughtered animals were collected for the determination of copper, manganese, zinc, iron, cadmium and molybdenum. The control group received a diet expected to result in a normal copper status. In order to induce an effective copper deficiency, the diet of the experimental group was supplemented with $CdSO_4$, $Na_2SO_4$ and $CaCO_3$. The control diet consisted of 42,4% oaten hay; 50% oats; 2,45% cane sugar; 1,25% urea; 1,9% $CaCO_3$; 1,6% NaCl and 3,7% $Na_2PO_4$; and the experimental diet of 39,8% oaten hay; 49% oats; 2,45% cane sugar; 1,25% urea; 5,27% $CaCO_3$; 1,47% $Na_2SO_4$; 0,0028% $CdSO_4$; 0,397% NaCl and 3,83% $Na_2PO_4$. Representative samples of the final rations as well as the liver samples were taken and prepared by wet acid digestion (van Ryssen & Stielau, 1980) for the determination of calcium, zinc, manganese, iron, cadmium and copper by atomic absorption spectrophotometry using a Philips Pye Unicam, model SP 9. Both phosphorous and molybdenum were determined spectrophotometrically according to the methods of the AOAC (1980) and Quin & Brooks (1975) respectively.

During the first part of the experiment, which lasted three months, blood samples were collected every second week for the determination of plasma copper. This was done by diluting 1 ml plasma 1:5 with double distilled water, after which it was aspirated directly into the atomic absorption spectrophotometer. Other blood constituents determined on a monthly basis during this time were phosphorous content (Goldenberg & Fernandez, 1966), glucose concentration (Hultman, 1959), blood urea nitrogen (Wybenga, Di Giorgio & Pileggi, 1971), plasma calcium (Gindler & King,1972), chlorine (Schales & Schales, 1941) and magnesium (Varley, Gowenlock & Bell, 1980). Potassium and sodium concentrations were determined by flame photometry. Albumin and total plasma protein content were determined by methods described by Tietz (1976). The globulin concentration was taken as the difference between the albumin and total plasma protein content. During this period the ewes were weighed monthly.

Following this period, the ewes were teased twice daily, in the morning and late afternoon, with vasectomized rams in order to identify those animals which came into oestrous. Heparinized blood samples were collected from each ewe in both groups for two consecutive oestrous cycles on days 1, 5, 8, 12 and 16 (day of oestrous) respectively, thereby covering all the developmental and functional phases of the *corpus luteum* during the oestrous cycle (Stabenfelt, Holt & Ewing, 1969). All blood samples were collected between 6h00 en 8h00, except in those cases where oestrous was detected in the afternoon. Blood samples were centrifuged as soon as possible after collection, and the plasma stored at –20°C until analysed for copper and progesterone. For all other parameters mentioned, analysis was normally completed within 2 days after collection. Total unconjugated progesterone was determined by radioimmuno-assay (van Niekerk & Morgenthal, 1982).

Following this phase, one fertile ram was introduced to each group while the animals still remained indoors in two separate groups receiving the two different diets. The rams were fitted with marker harnesses and marking blocks (Milborrow & Co.). Every 3rd day the rams were changed between the two groups. After ewes were served they were placed into individual pens while continuing to receive the same diet. After all the ewes in both groups were served once, the rams were removed. The ewes were kept on the same treatment for one month thereafter. They were then placed on kikuyu grazing where they additionally received 0,4 kg oaten hay and 0,15 kg barley, daily. Approximately 6 weeks before lambing started they were supplemented with an additional 0,2 kg of lucerne hay per day.

Haemoglobin typing was done by Prof. D.R. Osterhoff, Faculty of Veterinary Science, Department of Zoötechnology, University of Pretoria.

## Results and Discussion

The number of sheep belonging to each haemoglobin type in each group is given in Table 1. The mineral concentrations of the final two diets are given in Table 2.

As seen in Table 3 there were no noteworthy differences in the plasma phosphate, calcium, chlorine, potassium, magnesium, sodium, blood glucose and

**Table 1** The numbers of each haemoglobin type in the two groups

| | Group | |
|---|---|---|
| Haemoglobin | Control | Experimental |
| AA | 3 | 2 |
| AB | 2 | 4 |
| BB | 5 | 4 |
| Total | 10 | 10 |

**Table 2** The concentration of minerals and nitrogen in the experimental diets

| | P | Ca | Na | N | Cu | Zn | Mn | Fe | Cd | Mo |
|---|---|---|---|---|---|---|---|---|---|---|
| Diet | g/kg | | | | mg/kg | | | | | |
| Control | 3,05 | 6,00 | 8,5 | 16,65 | 2,65 | 16,28 | 46,5 | 40,82 | – | 0,137 |
| Experimental | 2,99 | 13,9 | 7,5 | 16,74 | 2,5 | 15,74 | 45,13 | 41,2 | 15,3 | 0,132 |

**Table 3** Effect of an induced copper deficiency on the plasma inorganic phosphate (P), calcium (Ca), chlorine (Cl), sodium (Na), magnesium (MG), potassium (K), blood urea nitrogen (BUN) and blood glucose concentrations during the first 12 weeks of the experiment

| | | P | Ca | Cl | Na | Mg | K | BUN | Glucose |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | Months | mmol/l | | | | | | | |
| Control | 0 | 1,78±0,27 | 2,25±0,12 | 109±1,97 | 144±1,56 | 0,69±0,09 | 4,41±0,27 | 4,59±0,92 | 3,2±0,19 |
| | 1 | 1,88±0,30 | 2,23±1,64 | 113±2,34 | 142±2,61 | 1,01±0,19 | 4,37±0,31 | 5,78±0,87 | 3,6±0,24 |
| | 2 | 1,74±0,20 | 2,60±0,16 | 108±2,21 | 142±2,76 | 0,80±0,07 | 4,38±0,22 | 7,82±0,93 | 3,5±0,32 |
| | 3 | 1,37±0,13 | 2,58±0,11 | 110±2,36 | 147±3,41 | 0,82±0,10 | 4,82±0,21 | 3,42±0,68 | 3,4±0,20 |
| Experimental | 0 | 1,86±0,29 | 2,27±0,14 | 110±2,19 | 145±2,59 | 0,68±0,07 | 4,46±0,24 | 3,36±0,96 | 3,4±0,24 |
| | 1 | 1,84±0,29 | 2,49±0,15 | 110±2,21 | 145±1,91 | 0,92±0,16 | 4,62±0,29 | 6,82±1,16 | 3,3±0,27 |
| | 2 | 1,67±0,19 | 2,45±0,15 | 107±1,81 | 143±3,34 | 0,75±0,08 | 4,34±0,23 | 7,30±1,07 | 3,6±0,29 |
| | 3 | 1,78±0,25 | 2,59±0,12 | 109±2,17 | 152±2,94 | 0,76±0,09 | 4,88±0,19 | 4,63±0,65 | 3,5±0,21 |

**Table 4** Total plasma concentrations (g/l) of protein (TPP), albumin and globulin during the first phase of the experiment

| | Group | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time in | Control | | | | Experimental | | | |
| months | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| TPP | 72,9±6,79 | 62,8±6,72 | 70,4±3,65 | 65,2±4,13 | 74,9±4,30 | 64,3±4,44 | 65,5±3,47 | 63,2±2,09 |
| Albumin | 31,8±3,76 | 31,6±4,32 | 31,4±1,83 | 36,3±2,71 | 31,7±1,25 | 33,1±2,51 | 31,0±1,63 | 35,4±2,11 |
| Globulin | 41,1±3,60 | 31,3±3,02 | 39,0±3,33 | 28,9±3,54 | 43,2±5,11 | 31,2±3,35 | 34,5±3,83 | 29,2±3,15 |

blood urea nitrogen concentrations between the two treatment groups during the first part of the experiment.

The total plasma concentrations of protein, albumin and globulin (Table 4) in the treated group were not affected if compared to that of the untreated group.

In the 12-week period during which the ewes received the experimental diets, the average plasma copper concentration decreased from 160 µg/dl to 56 µg/dl. In comparison, the average plasma copper values of the control group showed less variation and never fell below 100 µg/dl.

The decrease in plasma copper was probably due to the combined antagonistic effects of cadmium, calcium and sulphate. One may readily assume that cadmium was responsible for the sharp decrease in plasma copper (Mills & Dalgarno, 1972). Sulphate administration, and sulphate in combination with molybdenum normally results in higher than normal total plasma copper levels when these copper antagonists are administered to sheep (Suttle, 1980).

At the end of this 12-week period the four wethers in the experimental group were slaughtered. Mineral concentrations in the livers of these animals were compared to those of control animals (Table 5) and suggested that these ewes may have suffered from a copper deficiency.

Wool growth also showed a loss in crimp during that period. Results comparing the plasma progesterone and copper concentrations are presented graphically in

**Table 5** The liver mineral concentrations (Vg/g dry matter) of the two groups of sheep fed the experimental diets

| | Group | |
|---|---|---|
| Mineral | Control ($n$ = 6) | Experimental ($n$ = 4) |
| Copper | 131,8 ± 22,1 | 34,0 ± 16,5 |
| Iron | 254,6 ± 51,2 | 313,5 ± 55,2 |
| Manganese | 12,3 ± 1,2 | 14,75 ± 1,25 |
| Zinc | 160,5 ± 20,8 | 135,5 ± 19,1 |
| Molybdenum | 5,88 ± 0,75 | 3,2 ± 0,8 |
| Cadmium | – | 33,25 ± 8,65 |

Figures 1 (control) and 2 (experimental). The plasma copper concentration was almost without exception above 100 µg/dl in the control group, and no correlation with plasma progesterone was evident.

However, in contrast with this observation, fluctuating plasma copper concentrations were found in the experimental group. Important is the peak levels of the two substances occurring simultaneously on approximately day 12 of each consecutive oestrous cycle during this study, i.e. the period where progesterone secretion rate is at its highest level during the luteal phase of the cycle (Stabenfelt, *et al.*, 1967). The lowest plasma copper concentrations in the experimental group

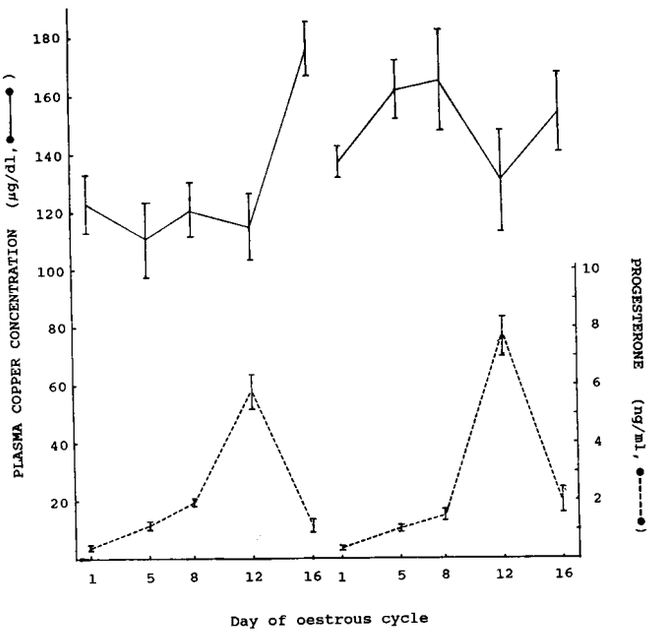


**Figure 1** A comparison between the mean plasma copper (± *SD*) and the mean plasma progesterone (± *SD*) levels on days 1, 5, 8, 12 and 16 of two consecutive oestrous cycles of the ewes in the control group

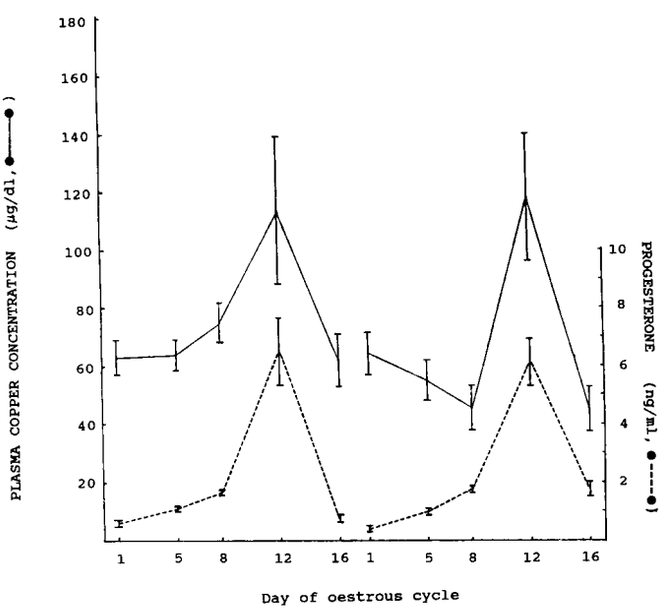


**Figure 2** A comparison between the mean plasma copper (± *SD*) and the mean plasma progesterone (± *SD*) levels on days 1, 5, 8, 12 and 16 of two consecutive oestrous cycles of the ewes in the experimental group

were recorded around oestrous, when the progesterone levels were at their lowest.

The fact that these experimental ewes showed clinical signs of a copper shortage, as evidenced by a loss of crimp in the wool, is important, demonstrating that the available copper was inadequate for normal body functions. By taking the results of the present study into consideration it seems that there may be an increased need for copper during the phase when progesterone

**Table 6** Average body mass (kg) of the two treatment groups during the first 12 weeks of the experiment

| Treatment group | Time in months | | | |
|---|---|---|---|---|
| | 0 | 1 | 2 | 3 |
| Control | 53,14 ± 4,92 | 57,40 ± 3,91 | 60,21 ± 5,23 | 64,01 ± 4,86 |
| Experimental | 52,91 ± 3,81 | 56,50 ± 3,62 | 59,49 ± 4,21 | 63,87 ± 4,83 |

production is at its highest. Although the reason for this rise in plasma copper is not clear, the conclusion can be made that the need for copper is higher for breeding ewes than barren animals.

The numbers of the animals used in the present study are too small to make any definite conclusion concerning lambing results. However, the lambing percentage of the ewes in the control group was 114%, and in the experimental group 120%. As seen in Table 6, the body mass of the ewes in both groups increased slightly during the course of the experiment.

It is well known that a chronic copper deficiency (in the long term) results in a decline in body mass. However, during the present study the time during which the animals suffered from a lowered copper status was probably too short to have any influence on body mass.

In areas marginally deficient in copper, where lustrous pasture growth occurs during spring, wool growth often shows a loss in crimp. Total plasma copper concentrations determined during this period usually suggest the existence of a copper deficiency. This transient copper deficiency is obviously due to a seasonal effect. In most of these cases, no adverse effects on body mass of adult sheep may be noticed, but when mating takes place during spring, the reproductive performance of ewes may be impaired (unpublished results). Observations made in an area where ewes suffered from a severe primary copper deficiency throughout the year, showed that animals were in a poor condition and that any attempt to breed such ewes would be unsuccessful (unpublished data). The effect which a transient copper shortage might have on the onset of oestrous after the anoestrous period should be of great relevance especially in those farming systems where enhanced mating frequency is practiced for fat lamb production.

In the light of these observations, as well as the results of the present study, it would seem that in cases where an animal suffers from a copper deficiency, a compensatory mechanism may be activated to regulate the circulating blood copper concentration during the time when the body is preparing for a possible pregnancy (luteal phase of the oestrous cycle). In this respect, the body copper reserves would play an important role. A practical implication of these results is that in cases where plasma copper values of less than 80 µg/dl (SA Mutton Merino) are observed, supplementary copper should be administered.

In the present study the copper distribution in the blood of the experimental animals was not determined.

Thus, it is not clear which fraction in the plasma was responsible for the subsequent rise in copper. The possible sources from which copper was mobilized during the luteal phase, as well as the actual mechanism involved in mobilizing the mineral and the possible role of copper in progesterone production are being further investigated.

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